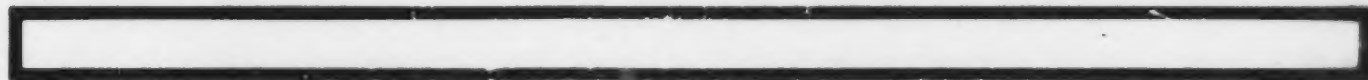


Two Acute Human Poisoning Cases
Resulting from Exposure to Diazinon
Transformation Products in Egypt

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Two Acute Human Poisoning Cases Resulting from Exposure to Diazinon Transformation Products in Egypt

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ABSTRACT. Two spraymen working in public health occupations in Alexandria, Egypt, experienced acute toxicity resulting from exposure to diazinon. Symptomatology was similar to that previously reported for exposure to parathion or other organophosphorus insecticides. Plasma and red blood cell cholinesterase activity values were determined in blood samples obtained from both individuals at various times after the incident. Cholinesterase activity showed a marked reduction up to 18 days after exposure. Blood cholinesterase activity recovered to approximately 90% of the normal level of activity 28 days after the poisoning incident in one individual. This activity recovered to about the same level in the other individual, but after only 20 days from the poisoning date. Experimental results suggested that this acute toxicity resulted from unsuitable storage conditions of the emulsifiable concentrate formulation of diazinon. The diazinon that was applied was stored in "tin" containers made of tin-plated sheet steel. The emulsifiable concentrate (EC) was not in compliance with the World Health Organization's standard specifications regarding the emulsion stability tests because of the presence of crystals in the emulsifiable concentrate. A sample of this crystalline material was analyzed. Gas chromatographic analysis combined with mass spectrometric techniques failed to identify intact diazinon in samples of that material. The sample represented virtually complete conversion of diazinon into transformation products. Sulfoteggs and monothionates were two of the identified products in the sample, both of which are much more toxic than diazinon.

DIAZINON, (O,O-diethyl O-[6-methyl-2-(4-methyl-ethyl-4-pyrimidin-2-yl) phosphorothioate] is an organophosphorus insecticide used widely to control insects of agricultural and public health significance in many countries around the world. It is formulated and distributed in several forms and it is known commercially by several names.¹ A number of fatal and nonfatal cases of accidental human diazinon intoxication have been reported.²⁻⁷ Chemistry, degradation, and toxicological properties of diazinon and its transformation products have been studied.⁸ Symptoms and treatment of diazinon poisoning have also been described.⁹

In Egypt, diazinon is used, at the recommendation of the Public Health Department of Egypt, to control cotton insect pests, as well as houseflies and mosquitoes. The active ingredient is imported from Ciba-Geigy Corporation of Switzerland and formulated as 60% emulsifiable concentrate (EC) at the Kair El-Zekai Insecticide Company in Egypt. No acute human poisoning incidents were reported during the application of this material during the last 3 yr. During that time, the formulations were packaged in 60% EC diazinon in aluminum cans pre-coated with inert material inside. To reduce the cost of the packaging process, the lin-

mulation company chose to distribute their new formulation of 60% EC diazinon in "tin" cans made of tin-plated sheet steel. The first batch of the new formulation was purchased in 1979 by the Public Health Department of Alexandria, Egypt. With the first use of the formulation in the "tin" containers, two human acute poisoning incidents were reported.

CASE REPORTS

Case 1. A 33-yr-old man had been spraying diazinon for more than 18 months. He used a back sprayer and did not use a mask or gloves. He sprayed approximately 1200-1500 L of diluted diazinon once a week during March through October. No sprays were applied during the winter.

On Saturday, April 14, 1979, this worker was spraying an area for housefly infestation in Seedy Beshr, Alexandria, with the new "tin" containers of 60% EC diazinon after the normal dilution (approximately 0.1%). Before the spraying was completed at noon the worker complained of nausea and vomiting. He became weaker and noted muscle twitching in both arms and legs. As he became more excited and breathing became more difficult, he was persuaded by his colleagues to come to the University Hospital, arriving at approximately 1:30 P.M. He was given 1 mg of atropine sulphate (intramuscularly) at approximately 2:30 P.M.; the dose was repeated after 2 hr. He remained at the hospital until the next day, at which time he was discharged without any other examinations or analyses.

Case 2. A 50-yr-old man had been spraying diazinon for more than 3 yr under the same conditions described for Case 1. He mentioned that on Thursday, April 12, 1979, he was spraying an area for housefly infestation in Moharam Bek, Alexandria, from the same new "tin" containers as did Case 1. In the afternoon, after completing the spraying operation, he complained of nausea and vomiting but went home as usual. He tried to eat dinner but vomited again. He later complained of burning eyes and blurred vision, as well as difficult breathing. As the evening progressed, he became weaker and his breathing became more difficult. From Friday through Sunday he had a severe headache but he did not leave his home. On Monday, April 16, he went to work and reported his illness.

On Monday, April 16, 1979, the director of Hygiene Department sent a report describing the incident along with a sample of the diazinon the two individuals had sprayed to the Pesticides Toxicology Lab and requested assistance on the examination of these cases and analysis of the insecticide sample.

Experimental. Blood samples (5 ml each) were drawn from Case 1 and 2 at approximately 11-wk intervals at 5 and 8 different occasions, respectively. Blood samples were also collected from 20 normal, unexposed male volunteers to serve as controls. Red and white blood cells (RBC and WBC, respectively) were counted using a Beckman-Coulter blood counter. Plasma and RBCs were isolated for determination of cholinesterase (ChE) activity using the Ellman techn-

que.¹⁰ Protein content of the plasma samples was determined by Lowry's method.¹¹

The two exposed individuals reported that the 1-L can containing 60% EC formulated diazinon from which they prepared the spraying emulsion was from a newly received batch of containers made of tin-plated sheet steel and not the regularly used aluminum cans. They also reported that when they used the material in the container crystals had formed in the formulation. One container from the same batch was submitted to our laboratory. The EC was tested according to the World Health Organization's (WHO) standard specifications regarding the emulsion stability tests.¹² A sample of the sedimented crystalline material from the formulation was stored in a dessicator at -10°C until transported and submitted for analysis by gas chromatography-mass spectrometry. This material was not subjected to any solvent washing or recrystallization.

Gas chromatography-mass spectrometry. Initial gas chromatography-mass spectrometry (GC-MS) studies were conducted using a Hewlett Packard 5930A mass spectrometer equipped with a model 5700A gas chromatograph (GC). Helium carrier gas flow rate was 42 ml/min. The membrane separator was located in the GC oven. Standard 70 eV conditions were used; filament 0.4 mA; electron multiplier 3 KV. The GC oven was programmed from 70°C (2 min) to 300°C at 8°C/min. A 183 cm x 2 mm [internal diameter (i.d.)] glass column packed with 1.5% OV-17/1.95% OV-210 on 80/100 mesh gas Chrom Q was used. Scanned mass spectra were observed on an oscilloscope and re-

Table 1.—Remaining Activity of Plasma and Red Blood Cell Cholinesterase at Different Times Following the Poisoning Incident

Time after Poisoning Incident (days)	% Remaining ChE Activity*			
	Case 1		Case 2	
	Plasma	RBC	Plasma	RBC
2	88.7	42.1	—	—
4	—	—	88.1	62.1
8	75.4	45.3	—	—
10	—	—	76.8	70.3
15	80.3	51.2	—	—
17	—	—	85.8	74.6
18	86.1	57.8	—	—
20	—	—	98.3	92.6
26	94.2	95.6	—	—

*Cholinesterase (ChE) activity as % of the corresponding average activity in plasma or RBC prepared from normal unexposed individuals. The normal plasma ChE activity was 2.0 ± 0.2 pmol of acetylthiocholine hydrolyzed/min-mg protein. The normal RBC ChE activity was $1.2 \pm 0.1 \times 10^3$ pmol of acetylthiocholine hydrolyzed/min-RBC.

corded, in rapid succession, on a light beam oscillograph.

A second study was performed on a Finnigan Corp. 4023 GC-MS with Inco's data system. A mass range of 46 to 470 m/z was scanned in 1.65 sec and the 70 eV mass spectra recorded with a filament emission of 0.40 mA and electron multiplier voltage of 1.5 KV. Two microliters of a solution of the crystalline sample in methylene chloride (2.92 mg/ml) were injected. The 12 M $\times$ 0.3 mm i.d., OV101, fused silica, Hewlett-Packard capillary GC column was directly connected into the ion source of the mass spectrometer and was programmed from 60°C (1 min) to 300°C at 8°C/min, with helium carrier gas flow rate of 1.5 ml/min. Experimental mass spectra were compared with those previously obtained from authentic standards and from a study of diazinon decomposition products.¹

RESULTS AND DISCUSSION

Blood cholinesterase activity. Red blood cell counts were 5.0 and 4.9 million cells/ μ l for Case 1 and 2, respectively (normal range: 4.0 to 7.0 million cells/ μ l). White blood cell counts were 9000 and 8400 cells/ μ l for

both cases, respectively (normal range: 5000 to 11000 cells/ μ l).

When the two cases were examined in our laboratory in Alexandria on Monday, April 16, 1979, they had a marked reduction in both plasma and RBC-ChE activity levels compared with those of normal, unexposed people. The values for RBC and plasma ChE in both patients are summarized in Table 1 for a comparable period after the diazinon exposure. As shown in Table 1, there was no substantial recovery in ChE values in Case 1. Within 2 wk, plasma ChE activity had substantially risen in each patient, but the improvement in RBC enzyme activity was minimal, especially in Case 1 up to 18 days after exposure.

Formulation tests. The contents of a container similar to those used by the two patients was examined for emulsion stability tests according to WHO standard protocols.¹² Observations and results indicated that the contents were not in compliance with the WHO standard specification. Separated crystallized material was found inside the container. The EC formulation did not give a stable emulsion during emulsion stability test. The two patients reported that they found similar crystals in the containers they used.

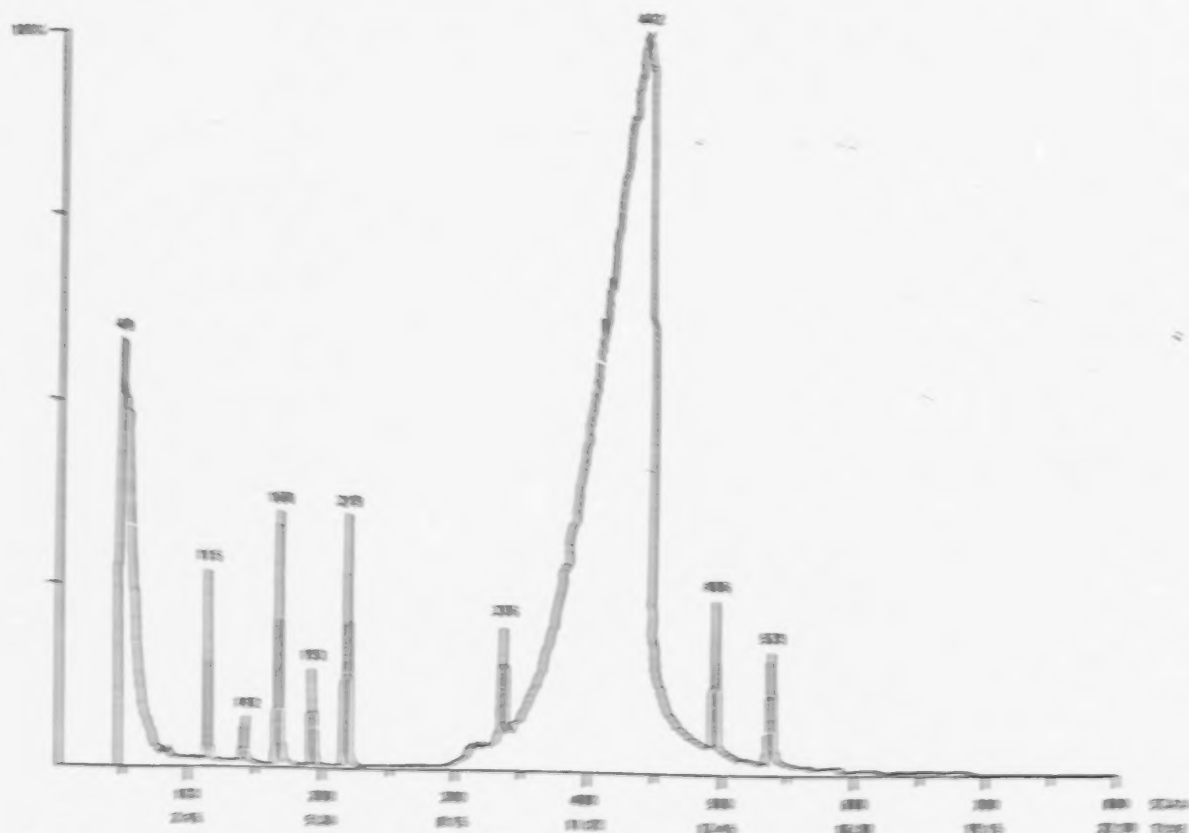


Fig. 1. Decomposition of diazinon (100 mg/ml) in methylene chloride. The 12 M $\times$ 0.3 mm GC-MS capillary column was programmed from 60°C (1 min) to 300°C at 8°C/min. The mass GC peaks are labeled as in their m/z number and are assigned in Table 2.

Analytical findings. Because the sample subjected for analysis was not prewashed or recrystallized, it is believed that the analysis should present information on the composition of the solid material as well as the surrounding liquid.

Examination of the sample by GC-MS led to the recognition of several chemical compounds. The reconstructed total ion chromatogram and structural assignments are provided in Figure 1 and Table 2, respectively. These results confirmed the chemical origin of the material from diazinon. The largest component of the mixture was the main hydrolysis product of diazinon, 7-isopropyl-4-methyl-6-hydroxypyrimidine. It exists principally in tautomeric forms: 6-methyl-2-(1-methyl-ethyl)-4(3H)-pyrimidinone and 6-methyl-2-(1-methyl-ethyl)-4(1H)pyrimidinone. The mass spectrum and chemical structures, respectively, are shown in Figure 2A.

Smaller amounts of other transformation products of diazinon were found: 4-ethoxy-6-methyl-2-(1-methyl-ethyl)-pyrimidine; 4-thioethoxy-6-methyl-2-(1-methyl-ethyl)-pyrimidine; 4,4'-thiobis[6-methyl-2-(1-methyl-ethyl)-pyrimidine]; 4,4'-dithiobis[6-methyl-2-(1-methyl-ethyl)-pyrimidine]; O,O,O-triethylphosphorothioate; and O,O,S-triethylphosphorothioate. Sulfotepp (O,O,O',O'-tetraethylthiopyrophosphate) was also found. The mass spectrum and chemical structure of sulfotepp are shown in Figure 2B. A second, later examination of the sample by GC-MS yielded, in addition, a mass spectrum assignable to a low level of monothionotetraethylpyrophosphate (monothiono-TEPP). The rapidly hydrolyzable tetraethylpyrophosphate (TEPP) could not be identified in the sample.

These products were similar to those detected in a North Carolina poisoning sample and are known transformation products of diazinon.^{1,7,8} These organophosphate products are of particular interest because they are the only known components of diazinon decomposition mixtures, with the possible exception of undetected species (diazoxon, s-ethyl diazinon, and dipyrimidinyl ethylphosphorothioate) with a potential for high human toxicity.^{6-8,13}

Marger and Gysin⁷ provided values for these compounds for the 50% inhibition of blood pseudocholinesterase in mg %, which when ratioed to diazinon itself, respectively, yielded values of: diazinon (0.8 mg %, 1.00 relative strength); dithionotetraethylpyrophosphate (2.9×10^4 mg %, 1,000); monothionotetraethylpyrophosphate, (5.5×10^5 mg %, 14,000); tetraethylpyrophosphate (9.7×10^5 mg %, 8,000). Thus, tetraethylpyrophosphate and di- and monothionotetraethylpyrophosphate are extremely potent acetylcholinesterase inhibitors.

Mammalian toxicity of these compounds is also reported to be very high. The acute oral LD₅₀ for rats is reported to be, respectively: sulfotepp (5 mg/kg); TEPP (0.2 to 2.0 mg/kg); and monothiono-TEPP (0.5 mg/kg); as compared to diazinon (300-850 mg/kg).¹³⁻¹⁶ Diazinon values show considerable variability in the literature due to different impurity levels of these very toxic deterioration products.¹⁶

Table 2.—Results of the Gas Chromatography-Mass Spectrophotometry Examination of the Poisoning Sample

Scan Number	Assignments
49	CH ₂ Cl ₂ Solvent
115	ArOCH ₃
142	ArOC ₂ H ₅
168	(C ₂ H ₅ O) ₂ p = O (SC ₂ H ₅)
193	(C ₂ H ₅ O) ₂ p = O
219	Ar SC ₂ H ₅
312	(C ₂ H ₅ O) ₂ p = O (O) p = S (OC ₂ H ₅) ₂ , Monothiono-TEPP + other components
336	(C ₂ H ₅ O) ₂ p = S (O) p = S (OC ₂ H ₅) ₂ , Sulfotepp
442	Ar OH
496	Ar-S-Ar
538	Ar-S-S-Ar

NOTE: Ar is the 6-methyl-2(1-methylethyl)-4-pyrimidinyl ring system.

Heat in the presence of acids has been shown to catalyze the decomposition of diazinon into these products.^{1,7} For this study it appears reasonable that the "tin" containers contributed to the catalytic decomposition of diazinon into these products. This may have been accompanied by the release of the toxic tetraethylpro- and thiopyrophosphate esters within a limited time period. Supporting this hypothesis were the facts that the individuals involved recognized the changed nature of their emulsifiable concentrate and that crystals precipitated in the formulation. Furthermore, the individuals had handled diazinon formulations for many years and had not developed any symptoms of poisoning until the aluminum containers were replaced with "tin" ones.

This class of tetraethyl-pyrophosphate and -mono and -di-thiono pyrophosphate esters has high vapor pressures and highly toxic vapors, and is rapidly absorbed through the skin.^{14,17} Obviously, both respiratory and dermal routes for poisoning are possible. These routes would be expected to be more prominent in higher temperature climates resulting from increased vapor pressures and faster rates of absorption.

These compounds are stabilized with respect to hydrolysis by the increasing number of thiono groups, therefore, the order of rate of hydrolysis is TEPP > monothiono-TEPP > sulfotepp.¹⁷ The finding of sulfotepp, a smaller level of monothiono-TEPP, and no TEPP in the sample is in accordance with their relative hydrolytic stabilities. In a sample of this kind, brought to a laboratory for investigation following an episode of poisoning, it is likely that the composition will vary due to different rates of formation of the toxic compounds by condensation and their destruction by hydrolysis. Thus, relating levels present in the sample to those at

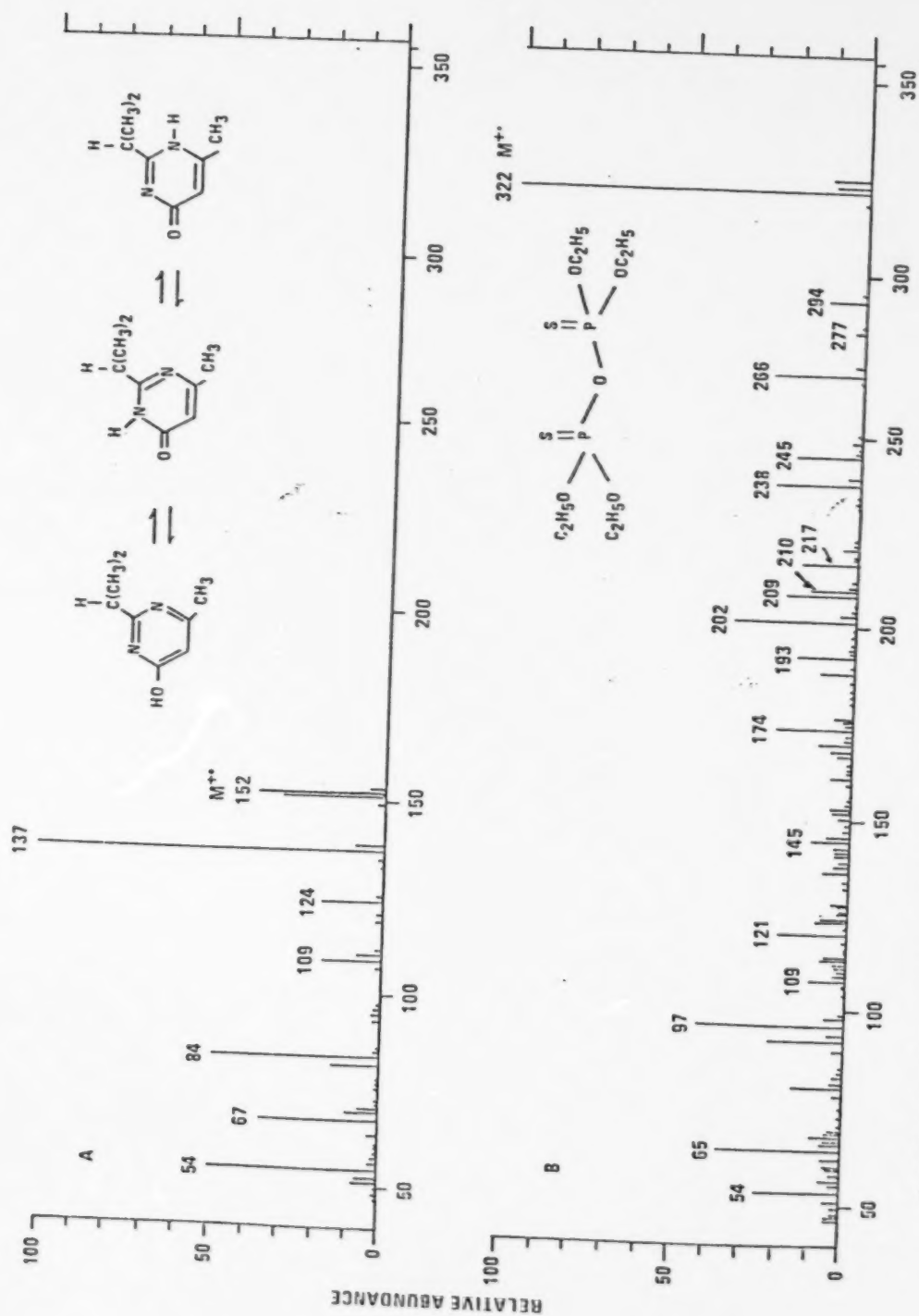


Fig. 2. Experimental mass spectra, shown with assigned chemical structures of two significant compounds found in the decomposed diazinon sample. (A) The main sample component, 2-isopropyl-4-methyl-6-hydroxypyrimidine, structure shown with the pyrimidinone tautomeric forms. (B) Mass spectrum of the most abundant, toxic, condensation product from the decomposed diazinon, Sulfotep, or dithionotetrathylpyrophosphate. M⁺ labels the molecular ion.

the time of the poisoning episode may be difficult. Finding in-tissue residues of these compounds may be difficult because of rapid hydrolysis and because relatively small levels are required to produce toxic effects. However, levels of diethylphosphate and diethylthiophosphate in urine would be expected to be elevated, and monitoring for these may prove useful. Particularly valuable, in this kind of poisoning, would be the finding of elevated organo-phosphate levels without elevated levels of 2-isopropyl-4-methyl-6-hydroxypyrimidine which would originate from the diazinon itself.¹⁸ This would clarify that it was the pyrophosphate esters and not intact diazinon that constituted the causative agent of the poisonings. Perhaps an increased awareness of possible poisoning by the transformation products will permit such analysis to be made.

CONCLUSION

This study supports the contention that diazinon transformation products may themselves be the

causative agents in certain diazinon-related poisonings. Thus, care in formulation, packaging, storage, and handling of diazinon solutions must be exercised to avoid generation of these toxic transformation products.

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